Isolation and Characterization of Cellulose from Rice Straw and Sugarcane Bagasse

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Declaration

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university/institution of higher learning.

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Table of Contents

List of Abbreviations

%	-	percent
^{O}C	-	degree celcius
h	-	hour
g	-	gram
mg	-	milligram
ml	-	mililitre
nm	-	nanometer
kV	-	kilovolt
min	-	minute
v / v	-	volume / volume
w / w	-	weight / weight
H_2O_2	-	peroxide
KBr	-	potassium bromide
SEM	-	Scanning Electron Microscopy
FTIR	-	Fourier Transform Infra Red

List of Table

List of Figures

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ABSTRACT

Cellulose from two types of lignocellulosic materials, rice straw and sugarcane bagasse were isolated by using two different methods, organosolv treatment and direct dissolution method. In organosolv treatment, solvent system consisted of a mixture of formic acid/acetic acid/water were used as these organic acids were proven to be the most effective solvents in delignification and removal of non-cellulose polysaccharides from the straw and bagasse, and do not have any undesirable side effects on cellulose properties. The bleaching step helped to degrade substantial amounts of residual hemicelluloses and lignin, which enabled the isolation of the cellulose samples of relatively high purity. For the direct dissolution method, a mixture of NaOH, thiourea and urea aqueous solution was used and hold at low temperature to enhance the dissolution power of cellulose. Isolated cellulose from both samples was characterized by SEM, FT-IR and CHN Analyzer.

Key words : Cellulose, lignocellulosic material, rice straw, sugarcane bagasse, organosolv treatment, direct dissolution method, hemicellulose, lignin.

ABSTRAK

Selulosa daripada dua jenis bahan berlignoselulosa, hampas padi dan hampas tebu telah diasingkan menggunakan dua kaedah yang berlainan, kaedah rawatan larutan organik dan kaedah penguraian terus. Dalam kaedah rawatan larutan organik, sistem larutan yang terdiri daripada campuran larutan asid formik/asid asetik/air telah digunakan memandangkan asid-asid organik ini telah terbukti sebagai pelarut yang efektif dalam delignifikasi dan penyingkiran polisakarida bukan selulosa daripada hampas tumbuhan, dan tidak mendatangkan kesan sampingan yang tidak diingini kepada sifat selulosa. Langkah pelunturan membantu menguraikan baki hemiselulosa dan lignin yang masih tertinggal, dan membantu pengasingan sampel selulosa yang tinggi ketulenannya secara relatif. Bagi kaedah penguraian terus, campuran larutan NaOH, thiourea dan urea telah digunakan dan dikekalkan pada suhu yang rendah untuk membantu penguraian lignin dan hemiselulosa. Selulosa yang telah diasingkan daripada dua sampel berkenaan telah dikarakteris menggunakan SEM, FT-IR dan penganalisa CHN.

Kata kunci : *Selulosa, bahan berlignoselulosa, hampas padi, hampas tebu, kaedah larutan organik, kaedah penguraian terus, hemiselulosa, lignin.*

CHAPTER 1

1.0 Introduction

Cellulose is one of the oldest natural polymers (Schurz, 1999). Nowadays, cellulose is known to have high potential applications in various fields. Because of its outstanding properties, it has attracted much attention as the most abundant renewable biopolymer in the world. It is renewable, biodegradable and can be derivatized to yield various useful products (Schurz, 1999). Cellulose also represents a vast potential feedstock for a number of industries and has created a great deal of research interest (Sun et al., 2004).

Cellulose is a polysaccharide, large molecule with hundreds or thousands of glucose molecules. It composed of β-(1→4)-linked D-glucopyranosyl units with three hydroxyl groups, which can form complex inter- and intra- molecular hydrogen bonds (Klemm, 2005). Cellulose is organized into fibrils, which are surrounded by a matrix of lignin and hemicellulose (Sun et al., 2004). In short, cellulose is found to be in combination with lignin and hemicellulose in all cellulosic materials. In order to obtain pure cellulose, it is important to isolate the cellulose from the cellulosic sample before we can use it for further reaction. The molecular linkages in cellulose form linear chains that are rigid, highly stable and resistant to chemical attack. Thus, cellulose cannot be dissolved in common solvents and does not melt before the thermal degradation. Many efforts have been made to identify suitable solvents for cellulose (Jin et al., 2007).

Researchers have developed various efficient methods for the isolation of cellulose from wood and other cellulosic materials (Sun et al., 2004). The cellulose isolation requires the removal of other substances such as hemicellulose and lignin from cellulosic samples. But, the problem is that the conventional methods used to isolate cellulose show various

disadvantages. For example, a protocol originally described by Green (1963) using acidified sodium chlorite is frequently applied to delignify cellulosic materials as an initial step in the isolation of cellulose, in which can cause serious environmental concerns. This shows that the isolation of cellulose fibres from agricultural residues requires degrading a large amount of lignin and hemicellulose and making the soluble in aqueous medium (Sun et al., 2004).

The processes currently employed for commercial straw pulping, which use inorganic reagents, achieved high cellulose extraction efficiency only at the expense of hemicellulose fractions, which undergoes hydrolysis and degradation. These processes can cause serious environmental problem. For these reason, intensive research is being carried out on the development of environmentally friendly approaches, which generally involves the use of organic solvents for efficient isolation of cellulose (Baeza et al., 1991; Vazquez et al., 1994).

So, it is important to study and find out the most effective method which can be used in cellulose isolation. The method must be more advantageous compared to the existing methods, which means more environmentally friendly, less chemical usage, low cost and time, high yield of cellulose and simple as well.

In this study, two methods were currently employed for the isolation of cellulose. The organosolv treatment and direct dissolution method will be applied for cellulose isolation of rice straw and sugarcane bagasse. By using two different methods, we can compare and find out the most efficient and advantageous method to isolate cellulose from both cellulosic sample. The effectiveness of the method used is largely depending on the type of cellulose sample. Each cellulosic material will give higher and better yield depending on the suitability with the method of cellulose isolation employed. For example, if the method of organosolv treatment is highly efficient for the sugarcane sample, it is not a must to say that this method is also highly efficient for the rice straw sample. In short, the amount of isolated cellulose

produced at the final stage is highly related to the method employed in cellulose isolation. Besides that, the isolated cellulose from both samples by using those methods will also differ in their physicochemical properties.

The interest in agricultural residues as an alternative source of cellulose fibres for the industry is growing, in part resulting from shortage of wood fibres and from agricultural overproduction in some countries of Asia (Moore, 1996; Shatalov et al., 2002). In this study, the rice straw is chosen as one of the lignocellulosic materials because of it overproduction in agricultural sector in Malaysia. There is a high amount of excessive rice straw that has not been used and is burnt at the final stage. It will be benefits to the industry if we can isolate the cellulose from rice straw and then regenerate the cellulose to produce various useful products in industry. The sugarcane bagasse is also chosen instead of rice straw. The significance of using two samples in this study is to compare the difference of cellulose yielded from those two lignocellulosic materials, by using same isolation method, organosolv pulping.

Objectives of Study

The objectives of this study include :

1. To isolate cellulose from rice straw and sugarcane bagasse samples
2. To characterize cellulose from rice straw and sugarcane bagasse samples
3. To compare the yield of cellulose isolated from two different cellulosic samples by two different isolation methods

CHAPTER 2

2.0 Literature Review

2.1 Cellulose

Cellulose is a polysaccharide with molecular formula of $(C_6H_{10}O_5)_n$. It is a linear polymer of anhydroglucose units linked at C-1 and C-4 by β-glycosidic bonds. This is confirmed by the presence of three hydroxyl groups with different acidity / reactivity, secondary OH at the C-2, secondary OH at the C-3, and primary OH at the C-6 position, and, accordingly, by the formation of strong various intermolecular and intramolecular hydrogen bonds (Kadla & Gilbert, 2000).

Cellulose is found in plants as microfibrils and can form the structurally strong framework in the cell walls. It is organized into fibrils, which is surrounded by a matrix of lignin and hemicellulose (Sun et al., 2003). Cellulose is mostly found in wood pulp. It is fabricated by many plants as hairs (cotton) or as a structural polymer in cells. It is also produced enzymatically by bacteria. Cellulose is also produced in a highly hydrated form by some bacteria (Schurz, 1999).

The cellulose fibril is partly crystalline, with two different crystal forms, cellulose Iα and cellulose Iβ. Cellulose Iα has one-chain triclinic structure and cellulose Iβ has two-chain monoclinic structure (Sugiyama, Vuong, and Chanzy, 1991), and they differ in hydrogen bonding (Sugiyama, Persson, & Chanzy, 1991). Cellulose Iα has been reported the dominant polymorph in bacterial and alga cellulose, while cellulose Iβ is predominant in higher plants such as cotton and wood. It is also known that cellulose Iα can be irreversibly converted to

cellulose Iβ by the application of heat (Atalla & VanderHart, 1984). This has also been noticed to happen during pulping (Newman & Hemmingson, 1995). Non-crystalline cellulose forms are also present in the fibril which are paracrystalline cellulose and cellulose at inaccessible and accessible fibril surfaces (Duchesne et al., 2001; Newman, 1998).

Cellulose is found in combination with the other components such as hemicellulose, lignin and ash in lignocellulosic material. The fractions of cellulose, hemicelluloses, and lignin obtained have different characteristics depending on the specific process conditions (Cordeiro et al., 2002). Cellulose fibre is suitable for either papermaking or enzymatic conversion to glucose, while hemicellulose sugars, can be used for various fermentation processes or as chemical feedstocks, and solid low-molecular weight lignin, is usable either as fuel or as feedstock for chemical conversions (Sarkanen, 1980). The molecular structure of cellulose is shown in Figure 1 below.

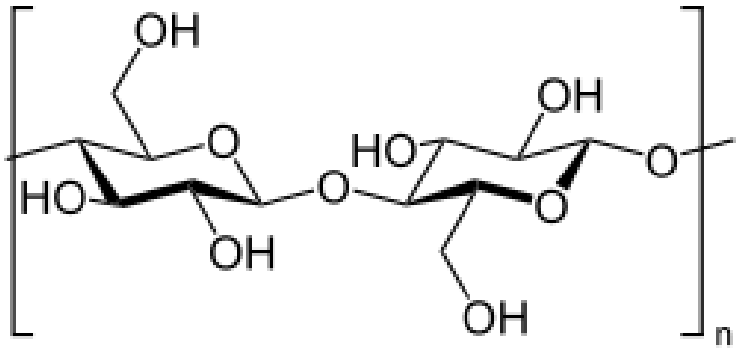

Figure 1 : Molecular Structure of Cellulose

2.2 Lignocellulosic Materials

Lignocellulose, a common biopolymer in the world (Juhász et al., 2005), is considered as one of the most important carbon sources on this planet (Nowak et al., 2005). Its degradation and subsequent utilization play an important role in the circulation of global carbon (Niranjane et al., 2007).

The structural materials that plants produce to form the cell walls, leaves, stems, stalks, and woody portions of biomass are composed mainly of three components called cellulose, hemicellulose, and lignin. Together, they are called lignocellulose, a composite material of rigid cellulose fibers embedded in a cross-linked matrix of lignin and hemicellulose that bind the fibers. Lignocellulose plant structures also contain a variety of plant-specific chemicals in the matrix, called extractives (resins, phenolics, and other chemicals), and minerals (calcium, magnesium, potassium, and others). They will leave ash when biomass is burnt (http://www.wisbiorefine.org/proc/fermlig.pdf).

Lignocellulose material is resistant to physical, chemical, and biological attack, but it is of interest to biorefining because the cellulose and hemicellulose can be broken down through a process called hydrolysis to produce simple sugars. Lignocellulosic biomass is often a waste material of the food processing and forest products industries that may be locally, readily available at low cost. Rice straw and sugarcane bagasse are two examples of lignocellulosic crop residues involved in this study.

2.3 Rice Straw

Rice straw, scientifically known as *Oryza sativa* L. is one type of lignocellulosic material. It is a biomass that contains lignocellulose components. Rice straw is produced in large quantities in many countries around the world. It is a major agricultural waste in rice-growing countries (Sun et al., 2008) and is one of the most abundant lignocellulosic crop residues (Sun et al., 2003).

From the data available, the quantity of rice straw produced per year in the world was probably about 570 million tones (Nguyen, 2000). With an increase in crop yields and cropping intensity, the management of rice by-products is becoming a problem as well as an opportunity. Rice straw is the residue and the excesses of production of rice that was not utilized. In traditional rice cropping systems, rice straw was either removed from the field at harvest time and stored as stock feed, or burnt in the field. As a result, the waste discharged can cause environmental problems and a loss of natural resources. If the wastes can be utilized, such as to enhance food production, they are no longer wastes but become new resources (Abdel-Mohdy, 2008).

At harvest, the moisture content of straw is usually more than 60% on a wet basis, however, in dry weather straw can quickly dry down to its equilibrium moisture content of around 10–12%. Rice straw has a high ash content (up to 22%) and low protein content (Abdel-Mohdy et al., 2008). The main carbohydrate components of rice straw are hemicellulose, cellulose and lignin. Rice straw consists of cellulose (35–40% w/w) and hemicellulose (25–30% w/w) in close association with lignin (10–15% w/w) (Thygesen et al., 2003). High silica content in rice straw (9–14%), however, prohibits the economic use.

It is clear from the collected data that the most traditional uses of rice by-products includes straw and hull for energy, animal feed, building materials and paper production (Nguyen, 2000). Traditionally, it was used as animal feed, feedstock for paper industry and organic fertilizer. However, most of the cellulose content in rice straw cannot be fully and economically utilized in these ways, in which can increase the environmental pollution problems subsequently. In recent years, environmental issues like reduction of carbon dioxide emission by blending bioethanol into gasoline, provide rice straw a new way to be utilized (Jørgensen and Olsson, 2006).

Table 1 : Classification of *Oryza sativa* L.

Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Liliopsida* – Monocotyledons
Subclass	*Commelinidae*
Order	*Cyperales*
Family	*Poaceae* – Grass family
Genus	*Oryza* L. – rice
Species	*Oryza sativa* L. – rice

2.4 Sugarcane Bagasse

Sugarcane is scientifically known as *Saccharum officinarum* L. Nowadays, sugarcane is widely used as an alternative fuel in industry. Consequently, the production of sugarcane is totally increasing due to this growing market.

According to Daniel et al. (2007), in spite of being good for business, this increasing in sugarcane production brings some environmental inconvenience, such as the generation of the huge amount of residues. For example, every metric ton of sugarcane generates about 280kg of bagasse. Some of this bagasse is burned to produce energy, but a huge amount is still not used. Since bagasse is composed mainly by cellulose (30-50%) and lignin (20-24%), many papers have been produced aiming at the utilization of bagasse cellulose for production of several cellulose derivatives (Liu et al., 2007; Pasquini et al., 2006; Vieira et al., 2007; Rodrigues et al., 2000) and bagasse lignin for production of phenolic resins (Khan et al., 2004; Pandey et al., 2000; Tita et al., 2002).

Table 2 : Classification of *Saccharum officinarum* L.

Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Liliopsida* – Monocotyledons
Subclass	*Commelinidae*
Order	*Cyperales*
Family	*Poaceae* – Grass family
Genus	*Saccharum* L. – sugarcane
Species	*Saccharum officinarum* L. – sugarcane

2.5 Methods of Cellulose Isolation

Various efficient methods have been developed by researchers in order to isolate cellulose from cellulosic materials. The cellulose isolation requires the removal of other substances such as hemicelluloses and lignin from wood and cereal straws. However, a protocol originally described by Green (1963) using acidified sodium chlorite is frequently applied to delignify wood as an initial step in the isolation of cellulose, which causes serious environmental concerns.

In other words, to obtain cellulose fibres from wood and agricultural residues, using traditional paper producing procedures, consists of degrading a large amount of lignin and hemicelluloses and making them soluble in the aqueous medium. For economically viable exploitation of this biomass, the first and important stage must be the efficient isolation of its major fractions which are cellulose, hemicelluloses, and lignin.

The processes currently employed for commercial straw pulping, which use inorganic reagents, achieve high cellulose extraction efficiency only at the expense of the hemicellulosic fraction, which undergoes hydrolysis and degradation. These processes not only underexploit the lignin, but also cause serious environmental problems. For these reasons, intensive research is being carried out on the development of environmentally friendly approaches, which generally involve the use of organic solvents for efficient separation of the three major components (Baeza et al., 1991 and Vazquez, 1994).

The organoslv treatment involves the treatment of lignocellulosic substances with organic solvent in water media in the presence or absence of a catalyst. Acetic acid pulping has been proved to be an effective organosolv method to delignify and fractionate wood and non-wood (Davis et al., 1986; Nimz and Casten, 1986; Pan and Sano, 1999 and Sano et al.,

1990). An advantage of delignification with acetic acid is that it can be followed immediately by bleaching, since addition of hydrogen peroxide yields the bleaching agent peracetic acid. Except for paper, the acetic pulp could be also used as raw material of cellulose derivatives because of the high content of cellulose (Uraki, Hashida, & Sano, 1997). Recently, one of the developments in acetic acid pulping is the Formacell process, based on the addition of 5–10% formic acid to aqueous acetic acid, resulting in improved selectivity of delignification (Lehnen, Saake, & Nimz, 2002). Besides their role in delignification, organic acids actively take part in the hydrolysis of hemicelluloses. Correspondingly, organic acid based pulping processes include the option for manufacture of dissolving pulps as a feedstock for cellulose derivatives and cellulosic fibres (Abad, Saake, Puls, & Parajo, 2002).

The direct dissolution method is another way to isolate cellulose from cellulose sample. More recently, Zhang and co-workers found that NaOH/urea and NaOH/thiourea aqueous solution can dissolve cellulose directly and quickly. Both solvent systems are inexpensive, less toxic and simple. However, spinning solutions containing high concentration of cellulose are unstable, which is a disadvantage in industrial applications. More over, the dissolution mechanism for cellulose in these solvent systems is not clear. A study conducted by Jin et al.,(2007) identified that a NaOH/thiourea/urea aqueous solution can dissolve quickly in direct dissolution method. They found that the new solvent they introduced is more powerful in dissolving cellulose, and can be used to prepare more stable spinning solutions containing higher concentrations of cellulose than aqueous solution used before. This method does not require activation treatment.

Besides these methods, the other methods that can be applied in cellulose isolation are the Jayme-Wise methods and Diglyme-HCl methods. According to the study of Cullen and Macfarlane, 2005, the Diglyme-HCl method leaves a small lignin residue in the crude

cellulose while in Jayme-Wise methods (Green, 1963), the α-cellulose produced is relatively pure. They conclude that the Diglyme-HCl method, with or without bleaching, appears to be a simple, fast method for extracting α-cellulose from hardwoods and softwoods.

2.6 Applications of Cellulose and Cellulose Derivatives

Cellulose is a linear polymer of anhydroglucose units linked at C-1 and C-4 by β-glycosidic bonds. This is confirmed by the presence of three hydroxyl groups with different acidity/reactivity, secondary OH at the C-2, secondary OH at the C-3, and primary OH at the C-6 position, and, accordingly, by the formation of strong various intermolecular and intramolecular hydrogen bonds (Kadla & Gilbert, 2000). The chemistry of cellulose is primarily the chemistry of alcohols. It forms many of the common derivatives of alcohols, such as esters, ethers, etc. These derivatives form the basis for much of the industrial technology of cellulose in use today.

Because of the strong hydrogen bonds that occur between cellulose chains, cellulose does not melt or dissolve in common solvents. Thus, it is difficult to convert the short fibers from cellulosic materials into the continuous filaments needed for artificial silk, an early goal of cellulose chemistry. Several different cellulose derivatives were examined as early routes to artificial silk, but only two, the acetate and xanthate esters, are of commercial importance for fibers today.

One of the most important cellulose derivatives is cellulose acetate, which is extensively used in several activities (coatings, membranes, cigar filters, etc.). One of its main applications nowadays is the production of membranes for separation processes such as hemodialysis, reverse osmosis and gas separation. Cellulose acetate is produced by cellulose acetylation, in

which cellulose reacts in the presence of acetic anhydride that is used as acetylating agent, acetic acid used as a solvent, and sulfuric acid or perchloric acid used as catalyst (Steinmeier, 2004, Sassi and Chanzy, 1995 and Edgar et al., 2001).

Cellulose acetate is soluble in organic solvents such as acetone and can be spun into fiber or formed into other shapes. Xanthate esters are formed when cellulose is first treated with strong alkali and then exposed to carbon disulfide. Cellulose xanthate is soluble in aqueous alkali and the resulting solution can be extruded as filaments or films. This is the basis for the viscose process for rayon manufacture (http://www.fibersource.com/f-tutor/cellulose.htm).

Recently, technology has been developed to form textile fibers (Lyocell) directly from wood pulp without using a derivative to facilitate dissolution. This technology is based on the ability of amine oxides, particularly N-methylmorpholine N-oxide, to dissolve unsubstituted cellulose. Except for paper, the acetic pulp produced by acetic pulping in this study could be also used as raw material of cellulose derivatives because of the high content of cellulose (Uraki et al., 1997).

Today cellulose is part of a number of common products. Rayon and cellophane are made by treating cellulose with an alkali and then exposing it to fumes of carbon disulfide, which yields threads and films. Cellulose acetates are used in photographic safety film and certain fabrics, and as a substitute for glass and a molding material. Cellulose ethers are used in paper sizings, adhesives, soaps, and synthetic resins. When mixed with nitric and sulfuric acids, cellulose forms flammable and explosive compounds, known as cellulose nitrates. Such nitrates include pyroxylin (found in lacquers and plastics), collodion (used in medicine, photography, and artificial leather manufacture) and guncotton, an explosive (http://www.bookrags.com/research/cellulose-wsd/).

CHAPTER 3

3.0 Materials and Methods

3.1 Sample Preparation

Cellulose was isolated from two different cellulosic materials, rice straw and sugarcane bagasse. The rice straw was obtained from Kuala Selangor, Selangor, while the sugarcane bagasse was collected from Kota Samarahan, Sarawak. The sugarcane bagasse was first cut into smaller pieces. Both rice straw and sugarcane bagasse samples were dried in an oven at 60^0C for 24 hours. This was to ensure that the raw samples were free from water molecule before being processed. The samples was ground by using a grinding machine (1mm) and stored in a drying cabinet at temperature of 32^oC until use to avoid fungus attack. All the other chemicals involved were used as received.

3.2 Isolation of Cellulose

Cellulose from both samples were isolated by using two methods which are the organosolv treatment method and direct dissolution method. For each method, both samples were processed simultaneously at one time. Yields of the cellulose obtained would be calculated on a dry weight basis related to the rice straw and sugarcane bagasse.